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LIGHT/TNFSF14 enhances adipose tissue inflammatory responses through its interaction with HVEM

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ABSTRACT

Obesity-induced adipose tissue inflammation is characterized by increased macrophage infiltration and cytokine production, and is associated with metabolic disorders. LIGHT/TNFSF14, a member of the TNF superfamily, plays a role in the development of various inflammatory diseases. The purpose of this study was to examine the involvement of soluble LIGHT (sLIGHT) in obesity-induced adipose tissue inflammatory responses. LIGHT gene expression on macrophages/adipocytes was upregulated by treatment with obesity-related factors. sLIGHT displayed chemotactic activity for macrophages and T cells, and enhanced inflammatory cytokine release from macrophages, adipocytes, and adipose tissue-derived SVF cells. The sLIGHT-induced inflammatory responses were blunted by neutralizing anti-HVEM antibody or knockout of HVEM, a receptor for sLIGHT. These findings indicate that sLIGHT enhances adipose tissue inflammatory responses through its interaction with HVEM.

1. Introduction

Obesity-induced inflammation is closely associated with the development of metabolic complications such as type II diabetes and atherosclerosis. Recent progress has led to the identification of several adipose tissue inflammatory components such as immune cells (e.g. macrophages/T cells) and cytokines/chemokines that account for obesity-induced inflammatory responses and insulin resistance [1–3]. However, the intrinsic factors that trigger adipose tissue inflammation are not yet completely understood.

LIGHT/TNFSF14 (lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpes virus entry mediator on T cells) is a member of the TNF superfamily (TNFSF) that binds to the membrane receptor HVEM/TNFRSF14 (herpes simplex virus glycoprotein D for herpes virus entry mediator). It is widely and constitutively expressed in various human and rodent tissues, and strongly expressed on resting and activated T cells, B cells, and monocytes [4]. The interaction between LIGHT and its receptor is involved in various inflammatory processes such as inflammatory bowel disease, rheumatoid arthritis and nephritis [5–8]. Recent studies have shown that soluble LIGHT (sLIGHT) promotes atherogenesis by inducing pro-inflammatory cytokines and matrix metalloproteinases in macrophage/foam cells and endothelial cells [9]. More recently, involvement of LIGHT (sLIGHT) and HVEM has been suggested in human obesity and metabolic disorders [10,11], but the mechanism by which sLIGHT mediates adipose tissue inflammatory responses remains elusive.

In this study, we demonstrate that sLIGHT plays a crucial role in adipose tissue inflammatory responses by enhancing macrophages/T-cell infiltration and activating the cells to release inflammatory cytokines. The inflammatory action of sLIGHT is mediated by interaction with its receptor HVEM. LIGHT and HVEM are potential targets for modulating obesity-induced adipose tissue inflammatory responses.

2. Materials and methods

See Supplementary material [19,20].

2.1. Statistical analyses

Data are presented as means ± S.E.M. Statistical analysis was performed using ANOVA and Duncan's multiple-range test. Differences were considered to be significant at $P < 0.05$.

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3. Results

3.1. LIGHT/HVEM gene expression in macrophages, adipocytes, and adipose tissue

To test whether LIGHT expression is associated with adipose tissue inflammatory responses in obesity, we treated macrophages and adipocytes with obesity-related factors such as free fatty acid (palmitate), oxidative stress (hydrogen peroxide), and adipose tissue-conditioned medium. Free fatty acid and oxidative stress enhanced LIGHT gene expression in 3T3-L1 adipocytes as well as Raw264.7 macrophages (Fig. 1A and B). Adipose tissue-conditioned medium prepared from obese mice also upregulated LIGHT gene expression in the macrophages (Fig. 1B). Expressions of HVEM gene were also stimulated by free fatty acid and/or adipose tissue-conditioned medium in adipocytes, macrophages, and adipose tissue-derived SVF cells (Fig. 1A–C).

We further examined whether the expression of HVEM or its ligand LIGHT in adipose tissue is altered in obese condition. C57BL/6 mice were fed a high-fat diet (obese mice) or a regular diet (lean mice) for 10 weeks, and using RT-PCR analysis the expression levels of HVEM and LIGHT in the adipose tissue of the obese and lean mice were measured. LIGHT mRNA level in adipose tissue was significantly higher in the obese mice than the lean control mice (Fig. 1D), and HVEM mRNA level was also upregulated in the obese mice (Fig. 1D). Additionally, we also examined coinhibitory receptors such as B and T lymphocyte attenuator (BTLA) and CD160 that bind to HVEM. Interestingly, BTLA mRNA level was significantly downregulated in the obese adipose tissue, but CD160 was not (Fig. 1E).

3.2. Chemotactic activities of sLIGHT for T cells and macrophages

Since the accumulation of T cells and macrophages in adipose tissue is crucial for adipose tissue inflammatory responses, we examined whether sLIGHT can induce macrophage/T cell infiltration. sLIGHT promoted the infiltration of Raw 264.7 macrophages (Fig. 2A) and peritoneal macrophages (Fig. 2B) as well as T cells (CD4+ and CD8+) (Fig. 2C) in a dose dependent manner. Adipose tissue-conditioned medium, which induced macrophage and T cell migration, was significantly more effective in inducing cell migration when sLIGHT was added (Fig. 2D). These findings suggest that adipose-derived sLIGHT may play a role in enhancing macrophage/T cell infiltration, and thus participate in adipose tissue inflammatory responses.

3.3. Effect of sLIGHT on cytokine/chemokine production by macrophages and adipocytes

To examine the effect of sLIGHT on inflammatory cytokine release from adipose cells, macrophages (Raw 264.7) and adipocytes (3T3-L1) were treated with sLIGHT and cytokine release was measured. sLIGHT treatment increased TNFα and IL-6 release from macrophages (Fig. 2E), MCP-1 and IL-6 from adipocytes (Fig. 2F), and MCP-1 and IL-6 from adipose tissue-derived SVF cells (Fig. 2G). This indicates that adipose tissue-derived sLIGHT may enhance inflammatory responses in obesity in an autocrine and/or paracrine manner.

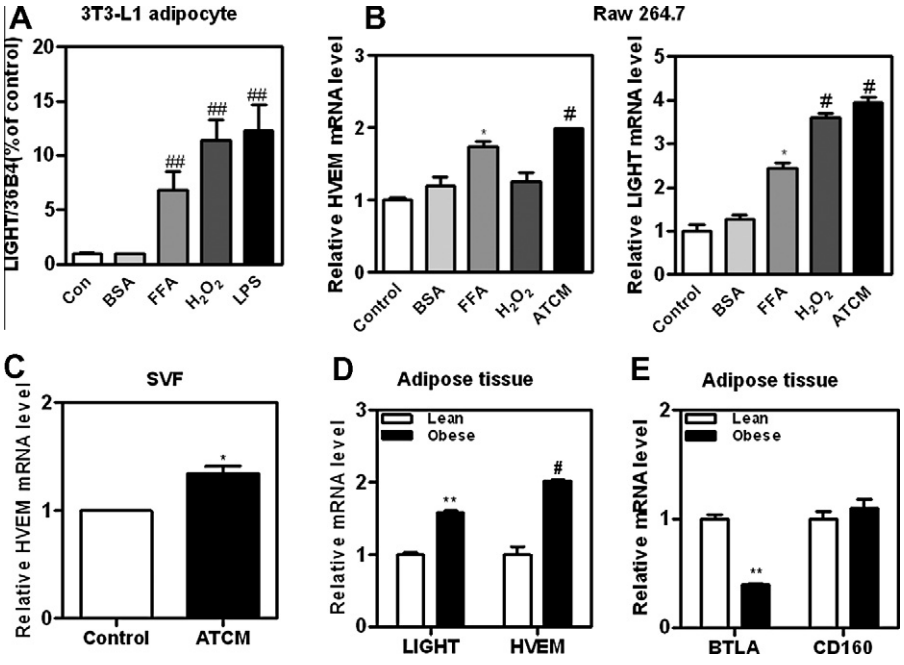

Fig. 1. Upregulation of LIGHT and/HVEM on macrophages and adipocytes by obesity-related factors and adipose tissue-conditioned medium. Macrophages (Raw 264.7), adipocytes (3T3-L1), and adipose tissue-derived SVF cells were treated with obesity-related factors (H_2O_2 50 μM, free fatty acid 500 μM) and/or adipose tissue-conditioned medium. Free fatty acid (palmitate) was prepared in ethanol containing bovine serum albumin (BSA, 50 μM). (A) LIGHT mRNA expression levels in adipocytes (3T3-L1) determined by semiquantitative reverse transcriptase-PCR analysis. 3T3-L1 adipocytes were treated with obesity-related factors for 24 h. Levels of mRNA were normalized to levels of 36B4 mRNA. Levels of HVEM or LIGHT mRNA in (B) macrophages or (C) adipose tissue-derived SVF cells were measured by a real-time reverse transcriptase-PCR analysis. Raw 264.7 macrophages were treated with obesity-related factors for 3 h. Levels of mRNA were normalized to levels of β-actin mRNA. Data represent results of three independent experiments. Values are means ± S.E.M. $^{*}P < 0.05$, $^{\#}P < 0.005$, $^{\#\#}P < 0.001$ compared to untreated control. Levels of (D) LIGHT, HVEM, (E) BTLA, and CD160 mRNAs in the epididymal adipose tissue of obese and lean mice. Levels of mRNA were estimated by real-time reverse transcriptase-PCR. Data are means ± S.E.M. of four animals for each group. $^{**}P < 0.01$, $^{\#}P < 0.005$ compared to the lean control.

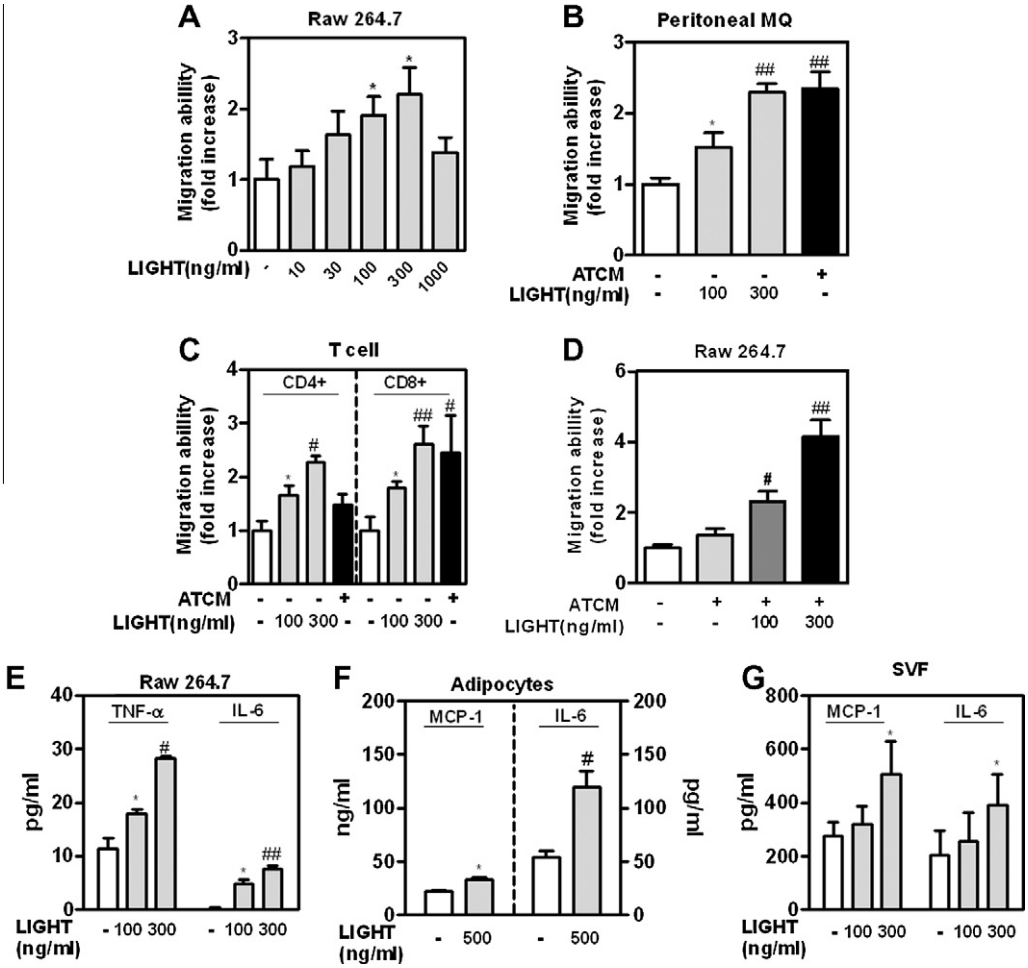

Fig. 2. sLIGHT-induced inflammatory responses of macrophages/T cells. Chemotactic activity was measured using a Boyden chamber. (A) Raw 264.7 macrophages (B) peritoneal MQs and (C) T cells (CD4+ and CD8+) were incubated for 3 h with sLIGHT (0, 100, and 300 ng/ml) and adipose tissue-conditioned medium (ATCM). (D) Macrophage (Raw 264.7) migration was induced by adipose tissue-conditioned medium (ATCM) containing sLIGHT (100 and 300 ng/ml). Cytokine concentrations in supernatants were determined by ELISA. (E) Raw 264.7 macrophages were incubated with 0, 100, and 300 ng/ml of sLIGHT for 18 h. (F) Mature 3T3-L1 adipocytes were incubated with 0, and 500 ng/ml of sLIGHT for 24 h. (G) Adipose tissue-derived SVF cells were incubated with 0, 100, and 300 ng/ml of sLIGHT for 18 h. Data represent results of three independent experiments. Values are means ± S.E.M. $^{*}P < 0.05$, $^{\#}P < 0.005$, $^{\#\#}P < 0.001$ compared to untreated control.

3.4. Effect of blockade and/or disruption of HVEM on sLIGHT-induced inflammatory responses

In order to test whether the sLIGHT-induced inflammatory effects are mediated by its receptor, HVEM, we examined the effect of neutralizing antibody against HVEM. As shown in Fig. 3A, sLIGHT-induced chemotactic activity for macrophages was blunted by exposure to anti-HVEM antibody. The chemotactic activity for macrophages induced by adipose tissue-conditioned medium was also inhibited by anti-HVEM antibody (Fig. 3B). We also examined the effect of HVEM deficiency on macrophages and T cells migration. As shown in Fig. 3C and D, sLIGHT-induced migration was markedly reduced in HVEM-deficient macrophages as well as T cells (CD4+ and CD8+). Moreover, sLIGHT-induced cytokine release (MCP-1 and IL-6) from adipose tissue-derived SVF cells was also markedly reduced (Fig. 3E and F). We also observed that MCP-1 and IL-6 production was impaired in HVEM-deficient SVF cells without sLIGHT treatment, and this may imply a role of HVEM in maintaining non-adipose cells homeostasis.

3.5. Cytokine release from adipocytes co-cultured with HVEM-deficient adipose tissue-derived SVF cells

When we co-cultured adipocytes with the adipose tissue-derived SVF cells from HVEM-deficient mice and wild type mice, the release of IL-6 and MCP-1 from the SVF cells was significantly lower in the case of the HVEM-deficient mice than in that of the wild type mice (Fig. 4A and B), indicating that adipocyte-derived LIGHT cannot act on SVF cells to cause release of cytokines in the absence of HVEM. These findings together strongly suggest that the LIGHT-induced inflammatory effects (e.g. chemotaxis and cytokine release) in adipose tissue are mediated by its interaction with HVEM.

4. Discussion

The present study demonstrates that the interaction between sLIGHT and HVEM contributes to adipose tissue inflammation by enhancing T cell/macrophage accumulation and activation, so creating a vicious inflammatory cycle.

The interaction between LIGHT and HVEM provides a pro-inflammatory signal to T cells and macrophages to produce inflammatory responses such as cytokine release, cell growth and survival [12–14]. Recent reports have shown that serum levels of sLIGHT increase in obese patients [11], and, moreover, sLIGHT increases release of inflammatory cytokines/chemokines from human adipocytes [11], suggesting a pathogenic potential for sLIGHT in obesity-induced inflammation. With regard to the cellular target of sLIGHT in adipose tissue inflammation, there are three

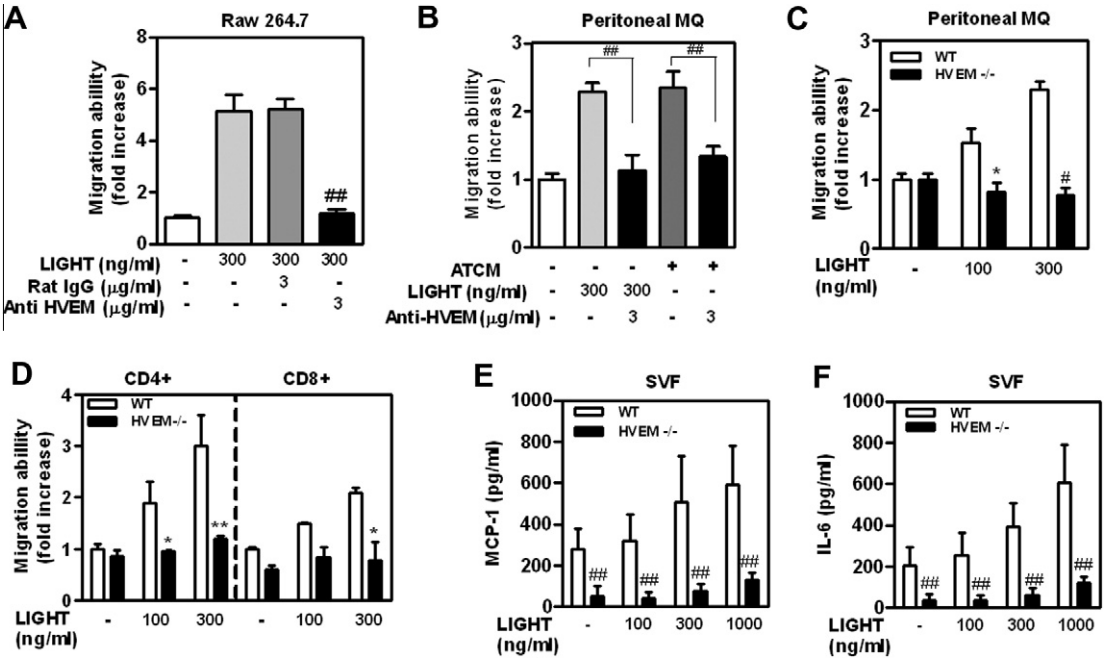

Fig. 3. Effect of anti-HVEM antibody and HVEM deficiency on sLIGHT-induced inflammatory responses. Macrophage were placed in the upper wells of a 96-well culture chamber separated from the lower wells containing sLIGHT or adipose tissue-conditioned medium, in the presence or absence of anti-HVEM mAb (1 and 3 μg/ml), and incubated for 3 h at 37 °C. Chemotactic activities of (A) Raw 264.7 cells and (B) peritoneal macrophages treated with anti-HVEM Ab. (C) Chemotactic activities of HVEM-deficient peritoneal macrophages (D) and T cells (CD4+ and CD8+) treated with sLIGHT. (E) sLIGHT-induced cytokine release. Adipose tissue-derived SVF cells isolated from HVEM-deficient mice were treated with sLIGHT for 18 h. Cytokine concentrations in the supernatant were determined by ELISA. Data represent results of three independent experiments. Values are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\#}P < 0.005$, $^{\#\#}P < 0.001$ compared to untreated control.

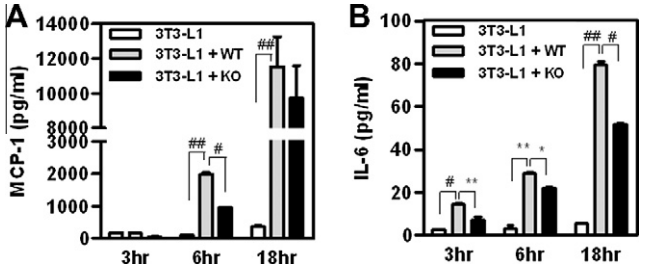

Fig. 4. Cytokine release from adipocytes co-cultured with HVEM-deficient adipose tissue-derived SVF cells. 3T3-L1 adipocytes (3×10^5 cells) were cultured in 24 well plates with or without adipose tissue-derived SVF cells (3×10^4 cells) from HVEM-deficient (KO) or wild type (WT) mice for 3, 6, and 12 h. (A) MCP-1 and (B) IL-6 cytokine concentrations in the supernatant were determined by ELISA. Data represent results of three independent experiments. Values are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\#}P < 0.005$, $^{\#\#}P < 0.001$ compared to 3T3-L1 adipocytes or 3T3-L1 adipocytes co-cultured with SVF cells from wild type mice.

possible components in adipose tissue: adipocytes, macrophages, and T cells. Thus, it was worth examining whether sLIGHT is involved in the crosstalk between T cells/macrophages and adipocytes that is crucial for adipose tissue inflammation. We observed first that LIGHT expression in macrophages and adipocytes was upregulated by obesity-related factors such as free fatty acid and oxidative stress. Continuously, we found that LIGHT and HVEM mRNA levels in adipose tissue were upregulated in obese mice, further underscoring the potential role of LIGHT in obesity-related adipose tissue inflammation. Given that sLIGHT enhances inflammatory responses in human monocytes [15], we hypothesized that the increase of sLIGHT in adipose tissue might stimulate adipose tissue macrophage recruitment and activation. Indeed, we found that sLIGHT had chemotactic activity for murine Raw 264.7 macrophages and peritoneal macrophages, and subsequently found that it can also induce T cells (CD4+ and CD8+) migration, indicating that sLIGHT could be a chemotactic factor released by adipose tissue enhancing macrophages/T cells accumulation. Moreover, we found that sLIGHT strongly stimulated release of inflammatory cytokines/chemokines (e.g. TNFα, IL-6, and MCP-1) from macrophages, adipocytes, and adipose tissue-derived SVF cells. Taken together, these findings suggest that sLIGHT released from adipose cells acts as a potent inducer of obesity-mediated adipose tissue inflammatory responses by recruiting T cell/macrophage into inflamed tissues and activating them, so aggravating adipose tissue inflammation (Fig. 5).

LIGHT binds to three distinct receptors: HVEM, lymphotoxin beta receptor, and decoy receptor 3; these are expressed on a variety of cell types, with the result that LIGHT elicits multiple cellular activities [16]. Upon binding to HVEM, it stimulates T cells/macrophages and accelerates their proliferation and cytokine production. Interestingly, it has been shown recently that HVEM, which is constitutively expressed on T cells and monocytes/macrophages [4], is markedly elevated in the adipose tissue of obese patients, and its expression is higher in visceral adipose tissue than in subcutaneous tissue [10]. HVEM expression has also been detected in mature human adipocytes and adipose tissue-derived SVF cells [10]. Although a role of the interaction of LIGHT with HVEM and other receptors in various inflammatory diseases has been suggested, the pathogenic effects and underlying mechanism in obesity-induced inflammation have not been fully explored. In view of the previously reported expression of LIGHT in adipose tissue [11] and the upregulation of HVEM and LIGHT by obesity-related factors observed by us, it seemed likely that LIGHT mediates adipose tissue inflammatory responses through HVEM. Indeed, treatment with neutralizing anti-HVEM antibody markedly blunted the infiltration of CD4+ or CD8+ T cells/macrophages and the release of cytokines induced by sLIGHT and adipose tissue-conditioned med-

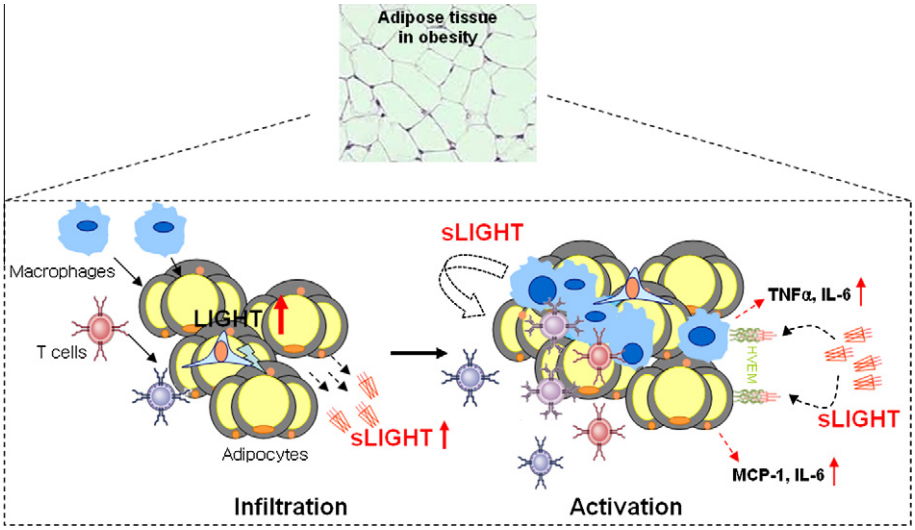

Fig. 5. Schematic presentation for the effect of sLIGHT adipose tissue inflammation. sLIGHT released from adipose cells acts as a potent inducer of obesity-mediated adipose tissue inflammatory responses by recruiting T cell/macrophage into inflamed tissues and activating them, so aggravating adipose tissue inflammation.

ium. In consistent with this, disruption of HVEM also blunted sLIGHT-induced migration of T cells/and macrophages and their cytokine production. These findings strongly support the idea that sLIGHT and HVEM interaction is important in promoting inflammatory responses in obese adipose tissue.

Additionally, it has been shown that HVEM can bind to coinhibitory receptors BTLA and/or CD160 [17]. The coinhibitory receptors transmit inhibitory signals bidirectionally, which complicates HVEM-mediated network [17]. However, it is likely that HVEM can simultaneously transmit inflammatory signal by activating NF-kB [18]. Blocking HVEM would then disrupt both costimulatory/coinhibitory signals. With respect this, we found that levels of LIGHT mRNA in adipose tissue was significantly increased in obese mice compared with lean control, whereas BTLA expression was markedly reduced, indicating that BTLA-mediated coinhibitory signal may be suppressed in obese conditions. Further study is needed to clarify how HVEM and its binding partners differentially participate in adipose tissue inflammation.

Taken together, these findings suggest that the HVEM/LIGHT interaction is important in promoting leukocyte infiltration/activation in obese adipose tissue, and that blocking HVEM or LIGHT may be useful for disrupting the pathogenic loop causing adipose tissue inflammation in obesity.

5. Conflicts of interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.01.011.

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